

WHAT IS CLAIMED IS

- Sub B1 5
1. A method of determining the apparent affinity (K_d) of binding between a PDZ domain and a ligand, comprising
- (a) immobilizing a polypeptide comprising the PDZ domain and a non-PDZ domain on a surface;
- (b) contacting the immobilized polypeptide with a plurality of different concentrations of the ligand;
- (c) determining the amount of binding of the ligand to the immobilized polypeptide at each of the concentrations of ligand;
- 10 (d) calculating the apparent affinity of the binding from the binding determined in (c).
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2. The method of claim 1, wherein the polypeptide is immobilized by binding the polypeptide to an immobilized immunoglobulin that binds the non-PDZ domain.
3. The method of claim 1, wherein the polypeptide comprising the PDZ domain is a fusion protein.
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4. The method of claim 3, wherein the fusion protein is a GST-PDZ domain fusion protein.
- Sub B2 25
5. A method of determining the K_i of an inhibitor or suspected inhibitor of binding between a PDZ domain and a ligand, comprising
- (a) immobilizing a polypeptide comprising the PDZ domain and a non-PDZ domain on a surface;
- (b) contacting the immobilized polypeptide with a plurality of different mixtures of the ligand and inhibitor, wherein the different mixtures comprise a fixed amount of ligand, at least a portion of which is detectably labeled, and different concentrations of the inhibitor;
- 30 (c) determining the amount of ligand bound at the different concentrations of inhibitor;
- (d) calculating the K_i of the inhibitor from the binding determined in (c).

6. The method of claim 5 wherein the polypeptide is immobilized by binding the polypeptide to an immobilized immunoglobulin that binds the non-PDZ domain.

7. The method of claim 6 wherein the fixed amount of ligand is between about 0.01 Kd and about 2 Kd.

8. A method of identifying an agent that enhances the binding of a PDZ domain to a ligand, comprising

(a) immobilizing a polypeptide comprising the PDZ domain and a non-PDZ domain on a surface;

(b) contacting the immobilized polypeptide with the ligand in the presence of a test agent and determining the amount of ligand bound; and,

(c) comparing the amount of ligand bound in the presence of the test agent with the amount of ligand bound by the polypeptide in the absence of the test agent,

wherein at least two-fold greater binding in the presence of the test agent compared to the absence of the test agent indicates that the test agent is an agent that enhances the binding of the PDZ domain to the ligand.

9. The method of claim 8 wherein the polypeptide is immobilized by binding the polypeptide to an immobilized immunoglobulin that binds the non-PDZ domain.

10. A method of determining the potency (K_{enhancer}) of an enhancer of binding between a PDZ domain and a ligand, comprising

(a) immobilizing a polypeptide comprising the PDZ domain and a non-PDZ domain on a surface;

(b) contacting the immobilized polypeptide with a plurality of different mixtures of the ligand and enhancer, wherein the different mixtures comprise a fixed amount of ligand, at least a portion of which is detectably labeled, and different concentrations of the enhancer;

(c) determining the amount of ligand bound at the different concentrations of enhancer;

(d) calculating the potency (K_{enhancer}) of the enhancer from the binding determined in (c).

11. The method of claim 10 wherein the polypeptide is immobilized by binding the polypeptide to an immobilized immunoglobulin that binds the non-PDZ domain.

12. The method of claim 11 wherein the fixed amount of ligand is between about
5 0.01 Kd and about 0.5 Kd.

13. A method of identifying a high specificity interaction between a particular PDZ domain and a ligand known or suspected of binding at least one PDZ domain, comprising:

(a) providing a plurality of different immobilized polypeptides, each of said
10 polypeptides comprising a PDZ domain and a non-PDZ domain;

(b) determining the affinity of the ligand for each of said polypeptides;

(c) comparing the affinity of binding of the ligand to each of said polypeptides,
wherein an interaction between the ligand and a particular PDZ domain
is deemed to have high specificity when the ligand binds an immobilized polypeptide
15 comprising the particular PDZ domain with at least 2-fold higher affinity than to immobilized
polypeptides not comprising the particular PDZ domain (a).

14. The method of claim 13 wherein the polypeptide is immobilized by binding the polypeptide to an immobilized immunoglobulin that binds the non-PDZ domain.

15. A method for determining the PDZ-PL inhibition profile of a compound
comprising:

(a) providing

(i) a plurality of different immobilized polypeptides, each of said
25 polypeptides comprising a PDZ domain and a non-PDZ domain;

(ii) a plurality of corresponding ligands, wherein each ligand
binds at least one PDZ domain in (i);

(b) contacting each of said immobilized polypeptides in (i) with a
corresponding ligand in (ii) in the presence and absence of a test compound;

(c) determining for each polypeptide-ligand pair in (b) whether the test
30 compound inhibits binding between the immobilized polypeptide and the corresponding ligand
thereby determining the PDZ-PL inhibition profile of the test compound.

16. An array comprising a plurality of different immobilized polypeptides, each of said polypeptides comprising a PDZ domain and a non-PDZ domain.

5 17. The array of claim 16 wherein the array is situated in a plastic multiwell plate.

18. The array of claim 16 comprising at least 12 different polypeptides comprising at least 12 different PDZ domains.

10 19. The array of claim 18 wherein the at least 12 different PDZ domains are from
PDZs expressed in lymphocytes.

20. The array of claim 19 wherein said PDZs are listed in Table 6.

15 21. An assay device comprising a plurality of different immobilized PDZ-containing
proteins organized in an array.

22. The device of claim 21 comprising at least 25 different PDZ-containing proteins.

23. A method for identifying an interaction between a PDZ domain and a PL comprising contacting a PL to a plurality of PDZ containing polypeptides and detecting binding of at least one PL to a PDZ.

24. The method of claim 23 wherein the contacting occurs on the assay device of claim 1.

25. The method of claim 23 wherein an interaction between a PDZ and more than one PL is detected.

26. The method of claim 23 wherein an interaction between a PL and more than one PDZ is detected.

27. A method for identifying a modulator of an interaction between a PDZ and a PL comprising carrying out the method of claims 23 in the presence and absence of a test compound and detecting a difference in at least one PDZ-PL interaction in the presence and absence of the test compound.

28. A method for identifying a modulator of an interaction between a PDZ and a PL comprising carrying out the method of claim 24 in the presence and absence of a test compound and detecting a difference in at least one PDZ-PL interaction in the presence and absence of the test compound.

29. The method of claim 27 wherein the modulator is an enhancer of the interaction.

30. The method of claim 27 wherein the modulator is an inhibitor of the interaction.